Individual and Interactive Effects of Temperature x Salinity on the Survival Rate of *Hemigrapsus sanguineus*

By

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Abstract

The *Hemigrapsus sanguineus*, or Asian shore crab, is an invasive crab that is currently invading rocky intertidal habitat on oceanic coasts across the northeastern United States. The species is thought to have been brought over via ship ballast water from its native range of the Eastern Asian shorelines. Asian shore crabs were first reported in North America in 1988 at Cape May, NJ; where they spread to their current invasive range from the coasts of North Carolina to Maine. The Asian shore crab's success as an invader has been attributed to its high reproductive rate, direct predation on competing crab species, superior competition for resources, and release from parasitism. Its tolerances of sub-optimal temperature and salinity may hamper the further spread of such a powerful invader is. Temperatures below 20°C and salinities below 25% have been shown through experiments to be unfavorable for survival rates of crabs collected from the NY coast of the Long Island Sound. My study found that Asian shore crabs have increased survival rates over x hours in cold water temperatures (12°C) when compared to 20°C and 27°C. I also find that as salinity decreases, survival rates decrease. The results of my study find that temperature and salinity likely each have their own individual effect on the survival of the crabs as well as an interactive, amplified, effect.

Introduction

Non-native species exist in a multitude of different ecosystems. As species cause ecological or economic harm in environments that are not a part of their native range, they can be considered invasive species (Pimentel et al., 2000). Coastal estuarine and marine ecosystems are currently among the most heavily invaded ecosystems in the world (Grosholz, 2002). These coastal and marine invaders can cause irreparable damage to the structure and functioning of the invaded ecosystems (Pimentel et al., 2000; Grosholz, 2002).

In the northeastern US, the *Hemigrapsus sanguineus,* or more commonly known as the Asian shore crab, is considered an invasive species. Asian shore crabs were introduced to this area prior to 1988 via ship ballast water, a common vector for marine invasive species with a larval state (McDermott 1991). Since their discovery at Cape May, NJ in 1988, Asian shore crabs have spread as far south as North Carolina and as far north as southern Maine (Stephenson et al., 2009). Further expansion northward into Canadian waters may be blocked by cold ambient temperatures (Stephenson et.al, 2009). However, as coastal waters warm, invasion opportunities could increase for cold limited species (Stephenson et al., 2009). The Asian shore crab is not believed to have reached its maximum southward range with southward expansion stalling around Cape Hatteras, NC. This halted expansion may be the result of habitat competition by native crab species or the lack of appropriate rocky habitat (McDermott, 1998).

Since its arrival in North America, *Hemigrapsus sanguineus* has become the dominant crab species in rocky intertidal habitat along much of the northeast coast of the U.S. (Epifanio, 2013). The Asian shore crab's success as an invader has been attributed to the species' high fecundity, direct predation on competing crab species, superior competition for space and food, and release from parasitism (Epifanio, 2013).

It is critical to know an invasive species' environmental tolerances for the potential use in the development of mitigation programs for the species as well for a prediction of the species' invasive range. When dealing with crabs, their temperature and salinity tolerances are two of the most important environmental tolerances to comprehend. The Asian shore crab is unable to persist in freshwater conditions (low salinity) therefore its distribution into estuaries is limited (Jungblut, 2015).

The goal of this study was to establish a matrix of temperature x salinity tolerances for *Hemigrapsus sanguineus* and to determine how the two variables affect the survival rates of the species on an individual and interactive basis. There were several hypotheses for this study: 1) Temperature and salinity would impact survival rates of the crabs at different magnitudes; with temperature being the stronger of the two stressors. 2) The crabs will have lower survival rates in less saline, warmer temperature environments; as these conditions are the opposite of the ones the species is typically found to be successful in. 3) Crabs would die more rapidly in warmer temperatures than in colder.

Materials and Methods

Hemigrapsus sanguineus samples were taken from the rocky beach at Read Wildlife Sanctuary, Rye NY (40.966040°N, 73.668391°W). One hundred crabs were collected during extreme low tides on September 21, 2019 and October 13, 2019. A 50-meter tape measure was set on the beach. First the tape measure was set parallel to the waterline, with the tape starting at the waterline. A random number generator was used to determine five points along the transect to collect crabs from. All rocks within 1 m^2 of the selected points were flipped over and all *Hemigrapsus sanguineus* under the rocks were collected by hand and placed into a cooler full of ice. The tape was then placed parallel to the waterline, 2m on shore from the waterline, where another 5 points were selected via random number generator and sampled. Transects were repeated until 100 viable crabs were collected on each sample day. Crabs under 10 mm in carapace width, over 25 mm in carapace width, and all gravid females were rejected and returned to the site.

Once the collection quota of 72 crabs was met, the crabs were placed in Ziploc bags and then placed in a cooler full of ice. Twenty-eight additional crabs were collected to serve as a buffer in case of stress mortality in transport. A mixture of *Fucus distichus,* more commonly known as rockweed, and *Fucus vesiculosus,* more commonly known as bladderwrack, was collected and placed in one Ziploc bag to act as food during the experiment. The samples were immediately transported to the lab.

Experimental Design

Thermal and saline environments were set up the night before sampling. Three different salinities were used: 35 ppt saltwater (standard ocean salinity), 12 ppt saltwater (approximately one-third standard ocean salinity), and 4 ppt saltwater (approximately one-ninth standard ocean salinity). The saltwater solutions were prepared using *Instant Ocean Salts* mixed with filtered water. Solutions were measured with a YSI salinometer before use. An oven was preset to 27° C (6°C above the September average sea surface temperature of 21°C for Rye, NY) while the lab bench was held at 20° C (1°C below the September average sea surface temperature of

21°C for Rye, NY). Samples were placed into 250 mL beakers in the sampling array denoted by Table 1. Sample beakers received 150 mL of their denoted treatment saltwater. Beakers were labeled with tape to indicate the environment set.

Experiment 1	Salinity (ppt)		
Temperature (°C)	4	12	35
20	12 beakers	12 beakers	12 beakers
27	12 beakers	12 beakers	12 beakers

Table 1: Treatment array for the September 21, 2019 sample group.

Immediately after the crabs arrived at the lab, they were removed from their bags and rinsed briefly with 35 ppt salinity water. Once rinsed, the crabs were tested for mortality, and then placed individually into the preset beakers if they passed the stimulus test. Mortality was determined via the following stimulus test: each crab was removed from its beaker and placed on its back atop the lab bench- if the crab could not right itself in under 2 seconds, it was considered functionally dead. All crabs were stimulus tested every 12 hours for seven days. The size and sex of each crab was recorded as well as their associated thermal x salinity environment. Crabs were fed a 15 mm piece of seaweed (soaked in 35 ppt salt water for 12 hours) at the 72-hour and 144-hour marks in the experiment. The water in the beakers was replaced every 24 hours with the same ppt of saltwater.

This experiment was repeated for the October 13, 2019 collection, with an incubator set to 12°C (6°C below the October average sea surface temperature of 18°C for Rye, NY); all other variables were held the same. The treatment array is denoted in Table 2.

Experiment 2	Salinity (ppt)		
Temperature (°C)	4	12	35
20	12 beakers	12 beakers	12 beakers
12	12 beakers	12 beakers	12 beakers

Table 2: Treatment array for the October 13, 2019 sample group.

Results:

Generally, survival declined over time in all treatments, though it declined faster for reduced salinity and for elevated temperature (Fig. 1). For the September sample group, the environment that showed the greatest mortality rate is the low salinity/elevated temperature (4 ppt / 27°C) treatment. This sample population declined down to one surviving crab after 48 hours, yielding a survival rate of 8% (Fig. 1, Fig. 3). The highest survival rate amongst the September sample group was the high salinity/low temperature (35 ppt / 20°C) treatment; with the sample population yielding a 48-hour survival rate of 83% and a 168-hour survival rate of 67% (Fig. 3, Fig. 4).

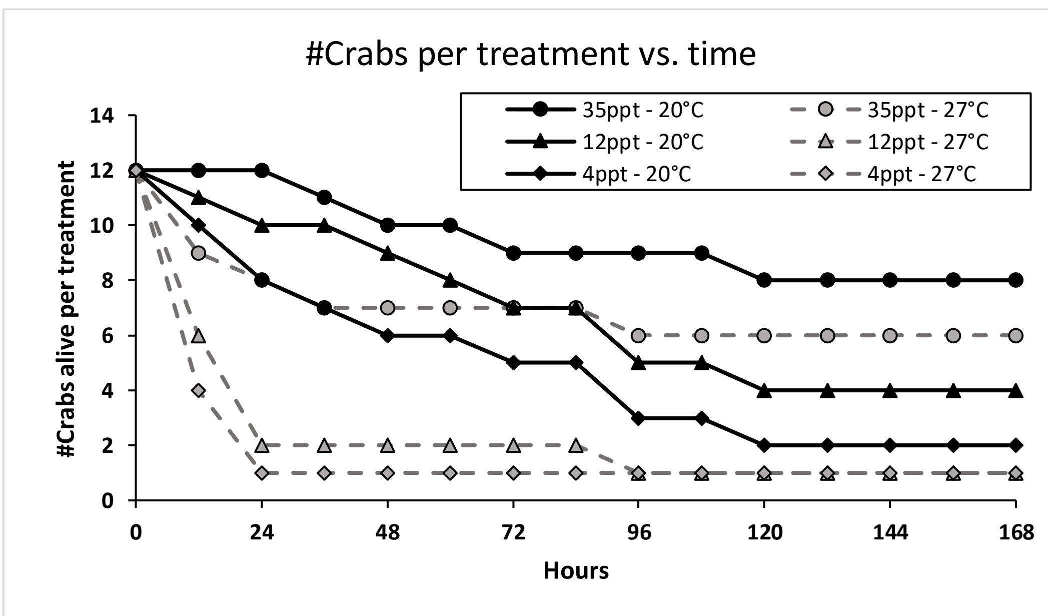

Figure 1: Number of crabs surviving per sample environment with respect to time for the September 13, 2019 sample. Temperatures tested were 20°C and 27°C. Salinities tested were 4 ppt, 12 ppt, and 35 ppt.

The environment amongst the October treatment group that showed the lowest 48-hour survival rate was the low salinity, higher temperature (4 ppt / 20°C) treatment; with a 33% survival rate (Fig. 2, Fig. 3). The environment with the highest 168-hour mortality rate was the low salinity, lower temperature (4 ppt / 12°C) treatment with a 17% survival rate (Fig. 2, Fig. 4).

The environments with the highest 48-hour survival rate are the high salinity/low temperature (35 ppt / 12°C), high salinity medium temperature (35 ppt / 20°C), and medium salinity/ medium temperature (12 ppt / 20°C) treatments; each yielding a 92% survival rate. (Fig. 3). The environment with the highest 168-hour survival rate is the high salinity, low temperature (35 ppt / 12°C) treatment, with a 92% survival rate (Fig. 4).

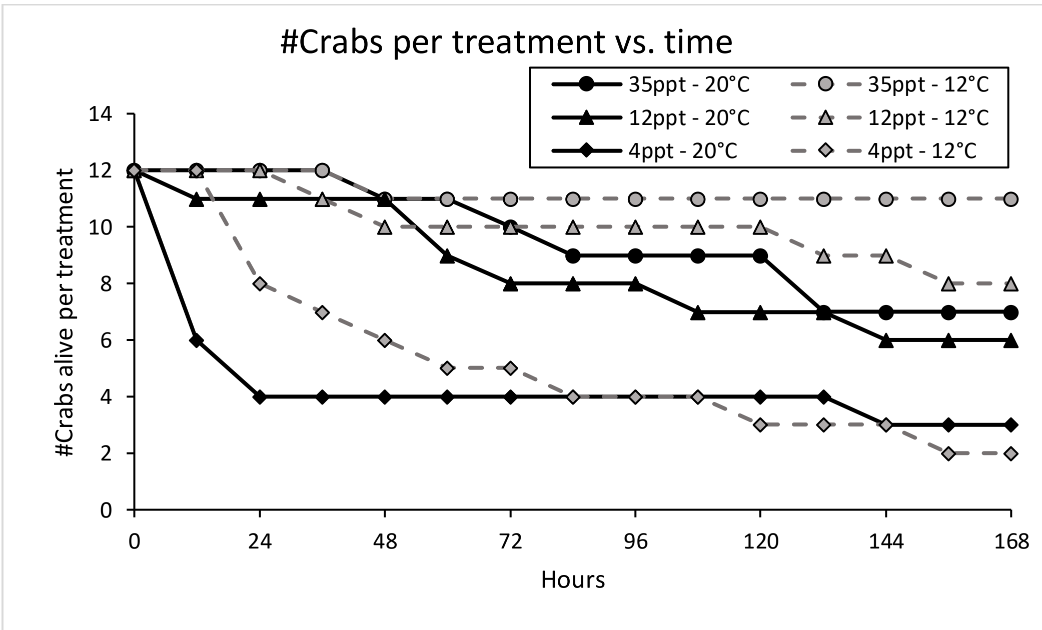

Figure 2: Number of crabs surviving per sample environment with respect to time for the October 13, 2019 sample. Temperatures tested were 20°C and 12°C. Salinities tested were 4 ppt, 12 ppt, and 35 ppt.

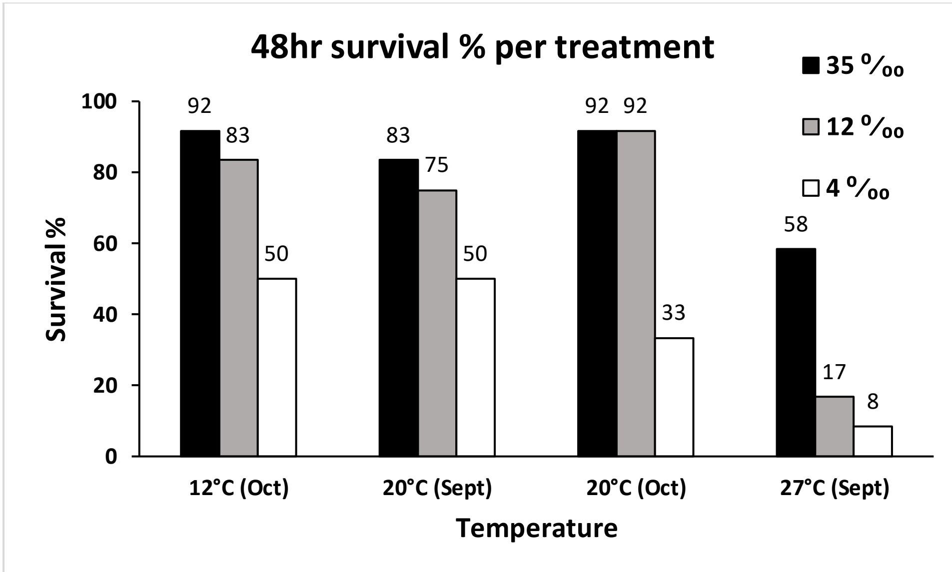

Figure 3: Survival rate of crabs in the first 48 hours of the experiment. Percentage of crabs surviving per treatment.

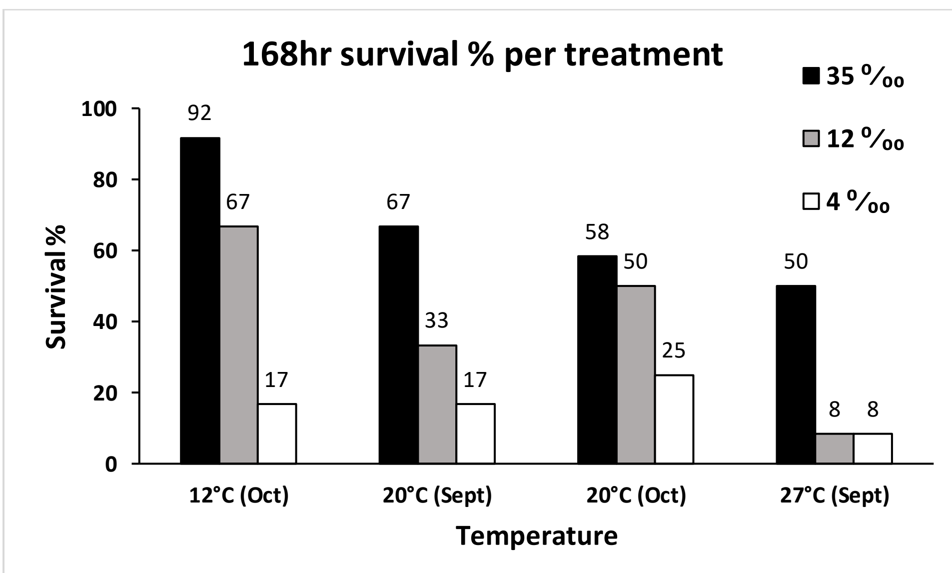

Figure 4: Survival rates of crabs over the 168 hours of the experiment. Percentage of sample surviving per treatment.

Discussion:

It is evident that salinity and temperature have both individual and interactive effects on the survival rate of *Hemigrapsus sanguineus.*

Individual Effects

As temperatures increase, survival rates generally decrease (Fig. 3, Fig. 4). Taking the average of the 48-hour survival rates with respect to temperature may allow for us to gauge the individual effect of temperature on short-term (48-hour) survival rates. In averaging the 48-hour survival rates for 12°C, 20°C, and 27°C (Fig. 3), the percentages in order, are as follows: 75%, 71%, and 28% survival rates. The 27°C average short-term survival rate is much lower than that of the 12°C and 20°C samples, indicating that 27°C is very likely an unfavorable temperature for the crabs' survival. The same calculations can be done for the 168-hour survival rates to gauge the long-term effects of temperature on survival rate. Taking the average of the 168-hour survival rates with respect to temperature the following numbers are produced: 59% survival for the 12°C samples, 42% survival for the 20°C, and 22% survival for the 27°C samples. A significant decrease in survival rates from 48-hours to 168-hours is evident across all temperatures. The highest temperate, 27°C, treatment had the lowest average survival rate over 168-hours so this temperature can likely be categorized as unsuitable for the species' survival.

Our individual temperature effects on survival rates directly contrast the findings of Stephenson et al. (2009) that the crabs' northern expansion is limited by cold temperatures. Their study states that the species is not found where summer mean sea surface temperatures fall below 12.6°C, but our study finds that the crabs' survive better 12°C thermal environments over all other tested thermal environments (20°C and 27°C) given that they had much higher survival rates in them. The study of Stephenson et al. (2009) makes this determination based on the species' native range on the eastern Asian coasts where mean summer sea surface temperatures range from 12.6°C at the northern end to 29°C at the southern end. Our study tested 12°C (just below the native temperature range), 20°C (in the middle of the native temperature range), and 27°C (on the high end of the native temperature range). The fact that my study shows that the crabs prefer the colder end of their native range and risk mortality at the higher end of the range may mean that the species has modified its tolerances to adapt to their new environment.

As salinity decreases, survival rates generally decrease over both 48-hour and 168-hour time spans (Fig. 3, Fig. 4). Taking the average survival rates of treatments with the same salinities, one may be able to gauge the individual effect of salinity on survival rates over 48-hours and 168-hours. Averaging the 48-hour survival rates presented in Fig. 3 with respect to salinity (omitting temperature), the following average rates are produced: 81% for 35ppt, 67% for 12 ppt, and 35% for 4 ppt. From these calculations, we can infer that 4 ppt salinity is likely unfavorable for the crabs to survive in over 48-hours. The same calculations can be done for the 168-hour salinities; producing average 168-hour survival rates of 67% for 35 ppt, 40% for 12 ppt, and 17% for 4 ppt (calculated using Fig. 4 data). Across all salinities, average survival rates decreased over time. The 4 ppt 168-hour average survival rate acts as strong proof that this salinity is unfavorable for the long-term survival of the crabs.

The findings of Epifanio et al. (1998) coincide well with our findings about the effects of salinity on the crabs' survival. Their tests determined that decreasing salinity from 30 ppt to 10 ppt generally decreases the survival rate of the crabs across all stages of life; our study finds the same trend when salinity decreases from 35 ppt to 4 ppt. These findings act as strong evidence that decreasing salinities will likely decrease the survival rates of the species.

Interactive Effects

48-hour (short-term)

High temperatures and low salinity increase the environmental stress on the *Hemigrapsus sanguineus* to the point where survival rates greatly decrease in the short term (time ≤ 48 hours) (Fig. 3). In the lowest saline and highest temperature (4 ppt / 27°C) treatment- eleven out of the twelve crabs died within 48 hours of the experiment's start; this is the most rapid short-term mortality rate out of all treatments with a survival rate of 8% (Fig. 3). In the medium saline and highest temperature (12 ppt / 27°C) treatment, ten out of the twelve crabs died within 48 hours- yielding a survival rate of 17% (Fig. 3). This is strong evidence to support that these combinations of environmental stressors are likely outside of the crabs' short-term (48 hour) tolerance range.

The low salinity / low temperature (4 ppt / 12°C) treatment, and both of the medium = low salinity / medium temperature (4 ppt / 20°C) treatments produced 48-hour survival rates between 33% and 50%; indicating that these matrices of environmental variables may be outside of the crab's short term tolerance range. This also ties the entirety of the 4 ppt salinity variable to 48-hour survival rates that may be or are likely outside of the species short term tolerance range; all of which having 48-hour survival rates of less than or equal to 50%. This strongly supports the idea that 4 ppt salinity (approximately one-ninth standard ocean salinity) is likely fatal to the species over 48 hours.

One of the medium salinity/medium temperature (12 ppt / 20°C) treatments and the high salinity/high temperature (35 ppt / 27°C) treatment yielded 48-hour survival rates between 50% and 75%; indicating that these combinations of variables may be within the crabs' tolerance range. The remainder of environmental treatment combinations: high salinity/low temperature, both of the high salinity/medium temperature, medium salinity/low temperature, and one of the medium salinity/medium temperature (35 ppt / 12°C, both 35 ppt / 20°C, 12 ppt / 12°C, and one of the 12 ppt / 20°C, respectively) all yielded 48-hour survival rates greater than 75%- indicating that these combinations of variables are likely within the species 48-hour tolerance range. Over the first 48-hours, the entirety of the 12°C treatments (approximately 6°C below October standard sea surface temperatures in Rye, NY) had survival rates of greater than 50%- indicating that this temperature may not be fatal to the crabs in the short term (48 hours). The same conclusion can be reached for the entirety of the 35 ppt treatments (standard ocean salinity) as they also all yielded 48-hour survival rates of greater than 50%.

168-hour (long term)

Over the long term (168 hours), survival rates decreased across all treatments. 168-hour survival rates of less than or equal to 25% were produced by the following treatments: low salinity/low temperature (4 ppt / 12°C), both of the low salinity/medium temperature (4 ppt /

20°C), the low salinity/high temperature (4 ppt / 27°C), and medium salinity/high temperature (12 ppt / 27°C). Given that these survival rates are less than or equal to 25%, it can be inferred that these environmental combinations are unfavorable for the crabs' survival and are likely outside of their tolerance zone. All of the 4 ppt treatments yielded said result, acting as strong evidence supporting that 4 ppt salinity (approximately one-ninth standard ocean salinity) is a significant stressor on the crabs' long term (7-day) survival.

Both of the medium salinity/medium temperature (12 ppt / 20°C) treatments and the high salinity/high temperature (35 ppt / 27°C) treatment yielded 168-hour survival rates of less than or equal to 50%; indicating that these combinations of variables may be outside of the crabs' tolerance range. The entirety of the 27°C temperature variable resulted in 168-hour survival rates of less than or equal to 50% indicating that this temperature may be unfavorable for the crabs' long term (7-day) survival.

Over 168 hours, both of the high salinity/medium temperature (35 ppt / 20°C) treatments and the medium salinity/low temperature (12 ppt / 12°C) treatment yielded survival rates between 50% and 75%. These higher survival rates may indicate that these combinations of variables are likely not as stressful as the combinations with long term survival rates of less than 50%. The remaining high salinity/low temperature (35 ppt / 12°C) treatment has a long-term survival rate of over 75% indicating that, of the combinations of variables tested, this combination was the most favorable for crab survival.

As salinity decreases and temperature increases, mortality rates increase (Fig.3, Fig. 4). This statement is partially backed by the findings of Epifanio et al. (1998) that show decreasing salinity from 30 ppt to 10 ppt yields an increase in mortality rates across all stages of the crabs' life. However, the findings of Epifanio et al. (1998) also show that increasing temperatures from 15°C to 25°C increases the survival rates amongst all stages in the crabs' life. Epifanio's results conclude that there was a strong effect of temperature on both survival and duration of larval development- coupled with the effects of salinity on survival rate; a strong interactive effect of the two variables can be observed in the survival rates of the crabs. This implies that the unfavorable temperatures and salinities, individual of one another, are stressful to the crabs.

The interaction, however, of the two variables' unfavorable states may amplify their stressful effects on the crabs, causing lower survival rates in unfavorable salinity/unfavorable temperature environments.

Possible Changes to Future Versions of the Experiment

This results of this experiment would benefit greatly from a much larger sample size. Increasing the number of crabs from 72 to 300 would likely increase the accuracy of the results. Additionally, increasing the number of collection dates from 2 (September and October) to 6 (May, June, July, August, September, October) may increase the likelihood of detecting seasonal variations in tolerances should they exist. Testing the survival rates of crabs for more than 7 days (perhaps 2 or 3 weeks) may help us to determine longer term survival trends. Increasing the number of tested temperatures from 3 to 5 (12°C, 16°C, 20°C, 24°C, and 28°C) would increase our understanding of how temperature effects survival rates. Increasing the number of salinities tested from 3 to 5 (4 ppt, 8 ppt, 12 ppt, 20 ppt, and 35 ppt) would provide us a greater understanding of how salinity effects survival rates. Applying all of the prospective changes at once, the ideal experiment becomes: six collections (once monthly from May-October) of 300 crabs distributed across a matrix of 5 different temperatures and 5 different salinities such that 12 crabs wind up in each thermal x saline environment with an experimental duration of 3 weeks.

Potential Application of Data

Oceanic warming occurs on a global scale and has already caused shifts in marine ecosystem composition and function (Portner, 2008). This increase in oceanic temperature provides increased invasion opportunity for potentially cold limited species like the *Hemigrapsus sanguineus* (Stephenson, 2009). Our study on temperature and salinity tolerance matrices for the *Hemigrapsus sanguineus* coupled with oceanic warming trends can be used to better focus the zones for invasive species management plans targeting *Hemigrapsus sanguineus* and to better predict the species' future range shifts. Zones with projected environmental variables that fall within fatal ranges for the crabs can have their efforts diverted to zones where their survival is favorable to provide the best chance of managing such a potent coastal invader.

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